	L #	Hits	Search Text	DBs
1	L1	395025	human	USPAT ; US-PG PUB
2	L2	25	RNASE ADJ H1	USPAT ; US-PG PUB
3	L3	18	L1 AND L2	USPAT ; US-PG PUB

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FILE 'REGISTRY' ENTERED AT 10:30:50 ON 05 NOV 2003

=> S RNASE H/CN
L1             1 RNASE H/CN

=> D

L1   ANSWER 1 OF 1  REGISTRY  COPYRIGHT 2003 ACS on STN
RN   9050-76-4  REGISTRY
CN   Nuclease, hybrid ribo- (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   E.C. 3.1.4.34
CN   Exoribonuclease H
CN   Hybrid nuclease
CN   Hybrid ribonuclease
CN   Hybridase
CN   Hybridase (ribonuclease H)
CN   Nuclease SSE1
CN   Nuclease, ribo-, H
CN   Ribonuclease H
CN   Ribonuclease H1
CN   Ribonuclease H2
CN   Ribonuclease HI
CN     ***RNase H***
CN   RNase H1
CN   RNase H2
CN   RNase HI
CN   RNase HII
CN   Structure-specific endonuclease 1
DR   64885-49-0, 37262-06-9
MF   Unspecified
CI   MAN
LC   STN Files:   AGRICOLA, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CAPLUS, CEN,
       CHEMCATS, CIN, CSCHEM, EMBASE, NAPRALERT, PROMT, TOXCENTER, USPAT2,
       USPATFULL

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
            1598 REFERENCES IN FILE CA (1907 TO DATE)
              22 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
            1604 REFERENCES IN FILE CAPLUS (1907 TO DATE)


FILE 'CAPLUS' ENTERED AT 10:31:26 ON 05 NOV 2003


=> S L1;S RNASE H;S RNASE (H1 OR H(W)1);S HUMAN;S MUTATION;S DELETION;S POINT
L2          1604 L1

          34381 RNASE
           2519 RNASES
          34937 RNASE
                  (RNASE OR RNASES)
        2386086 H
L3         2614 RNASE H
                  (RNASE(W)H)


=> S RNASE (W) (H1 OR H(W)1);S HUMAN;S MUTATION;S DELETION;S POINT
          34381 RNASE
           2519 RNASES
          34937 RNASE
                  (RNASE OR RNASES)
          21271 H1
        2386086 H
        7718253 1
L4           79 RNASE (W) (H1 OR H(W)1)


        1192793 HUMAN
         310564 HUMANS
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..      ..
L5      1352471 HUMAN
                (HUMAN OR HUMANS)


         192679 MUTATION
         123808 MUTATIONS
L6       238918 MUTATION
                (MUTATION OR MUTATIONS)


          70783 DELETION
          22725 DELETIONS
L7        81867 DELETION
                (DELETION OR DELETIONS)


         536586 POINT
         165255 POINTS
L8       668096 POINT
                (POINT OR POINTS)

=> S L5 AND L3;S L5 AND L4
L9          807 L5 AND L3


L10          22 L5 AND L4

=> D 1-22 CBIB ABS

L10  ANSWER 1 OF 22  CAPLUS  COPYRIGHT 2003 ACS on STN
2003:764454  Selective inhibitory DNA aptamers of the   ***human***
       ***RNase***     ***H1***  .  Pileur, Frederic; Andreola, Marie-Line;
     Dausse, Eric; Michel, Justine; Moreau, Serge; Yamada, Hirofumi;
     Gaidamakov, Sergei A.; Crouch, Robert J.; Toulme, Jean-Jacques; Cazenave,
     Christian (IFR Pathologies Infectieuses, Universite Victor Segalen
     Bordeaux 2, INSERM U386, Bordeaux, 33076, Fr.).  Nucleic Acids Research,
     31(19), 5776-5788 (English) 2003.  CODEN: NARHAD.  ISSN: 0305-1048.
     Publisher: Oxford University Press.
AB     ***Human***     ***RNase***     ***H1***   binds double-stranded RNA via
     its N-terminal domain and RNA-DNA hybrid via its C-terminal RNase H
     domain, the latter being closely related to Escherichia coli RNase HI.
     Using SELEX, we have generated a set of DNA sequences that can bind
     efficiently (Kd values ranging from 10 to 80 nM) to the   ***human***
       ***RNase***     ***H1***  .  None of them could fold into a simple perfect
     double-stranded DNA hairpin confirming that double-stranded DNA does not
     constitute a trivial ligand for the enzyme.  Only two of the 37 DNA
     aptamers selected were inhibitors of   ***human***     ***RNase***
       ***H1***   activity.  The two inhibitory oligomers, V-2 and VI-2, were
     quite different in structure with V-2 folding into a large, imperfect but
     stable hairpin loop.  The VI-2 structure consists of a central region
     unimol. quadruplex formed by stacking of two guanine quartets flanked by
     the 5' and 3' tails that form a stem of six base pairs.  Base pairing
     between the 5' and 3' tails appears crucial for conferring the inhibitory
     properties to the aptamer.  Finally, the inhibitory aptamers were capable
     of completely abolishing the action of an antisense oligonucleotide in a
     rabbit reticulocyte lysate supplemented with   ***human***     ***RNase***
       ***H1***  , with IC50 ranging from 50 to 100 nM.

L10  ANSWER 2 OF 22  CAPLUS  COPYRIGHT 2003 ACS on STN
2003:346658  Document No. 139:130349  MODIP revisited: re-evaluation and
     refinement of an automated procedure for modeling of disulfide bonds in
     proteins.  Dani, Vardhan S.; Ramakrishnan, C.; Varadarajan, Raghavan
     (Molecular Biophysics Unit, Indian Institute of Science, Bangalore,
     560012, India).  Protein Engineering, 16(3), 187-193 (English) 2003.
     CODEN: PRENE9.  ISSN: 0269-2139.  Publisher: Oxford University Press.
AB   There have been several attempts to stabilize proteins through the
     introduction of engineered disulfide bonds.  For reasons that are
     currently unclear, these have met with mixed success.  Hence
     identification of locations where introduction of a disulfide cross-link
     will lead to protein stabilization is still a challenging task.  A
     computational procedure, MODIP, was introduced more than a decade ago to
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select sites in protein structures that have the correct geometry for disulfide formation when replaced by Cys. In this study, we re-evaluated the stereochem. criteria used by MODIP for the selection and gradation of sites for modeling disulfides. We introduced steric criteria to check for energetically unfavorable non-bonded contacts with the modeled disulfide, since these can considerably offset the stabilizing effect of the cross-link. The performance of the refined procedure was checked for its ability to correctly predict naturally occurring disulfide bonds in proteins. A set of proteins in which disulfide bonds were introduced exptl. were analyzed with respect to MODIP predictions, stability and other parameters such as accessibility, residue depth, B-factors of the mutated sites, change in vol. upon mutation and loop length enclosed by the disulfide. The anal. suggests that in addn. to proper stereochem., stabilizing disulfides occur in regions of low depth, relatively high mobility, have a loop length greater than 25 and where the disulfide typically occupies a vol. less than or equal to that of the original residues.

L10 ANSWER 3 OF 22 CAPLUS COPYRIGHT 2003 ACS on STN
2003:304591 Document No. 139:129797 ***Human*** ***RNase*** ***H1*** Activity Is Regulated by a Unique Redox Switch Formed between Adjacent Cysteines. Lima, Walt F.; Wu, Hongjiang; Nichols, Josh G.; Manalili, Sherilynn M.; Drader, Jared J.; Hofstadler, Steven A.; Crooke, Stanley T. (Department of Molecular and Structural Biology, Isis Pharmaceuticals, Carlsbad, CA, 92008, USA). Journal of Biological Chemistry, 278(17), 14906-14912 (English) 2003. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB ***Human*** ***RNase*** ***H1*** is active only under reduced conditions. Oxidn. as well as N-ethylmaleimide (NEM) treatment of ***human*** ***RNase*** ***H1*** ablates the cleavage activity. The oxidized and NEM alkylated forms of ***human*** ***RNase*** ***H1*** exhibited binding affinities for the heteroduplex substrate comparable with the reduced form of the enzyme. Mutants of ***human*** ***RNase*** ***H1*** in which the cysteines were either deleted or substituted with alanine exhibited cleavage rates comparable with the reduced form of the enzyme, suggesting that the cysteine residues were not required for catalysis. The cysteine residues responsible for the obsd. redox-dependent activity of ***human*** ***RNase*** ***H1*** were detd. by site-directed mutagenesis to involve Cys147 and Cys148. The redox states of the Cys147 and Cys148 residues were detd. by digesting the reduced, oxidized, and NEM-treated forms of ***human*** ***RNase*** ***H1*** with trypsin and analyzing the cysteine contg. tryptic fragments by .mu. high performance liq. chromatog.-electrospray ionization-Fourier transform ion cyclotron mass spectrometry. The tryptic fragment Asp131-Arg153 contg. Cys147 and Cys148 was identified. The mass spectra for the Asp131-Arg153 peptides from the oxidized and reduced forms of ***human*** ***RNase*** ***H1*** in the presence and absence of NEM showed peptide masses consistent with the formation of a disulfide bond between Cys147 and Cys148. These data show that the formation of a disulfide bond between adjacent Cys147 and Cys148 residues results in an inactive enzyme conformation and provides further insights into the interaction between ***human*** ***RNase*** ***H1*** and the heteroduplex substrate.

L10 ANSWER 4 OF 22 CAPLUS COPYRIGHT 2003 ACS on STN
2003:303837 Document No. 139:2874 Expression of RNase H of ***human*** hepatitis B virus in Escherichia coli. Cheng, Hong; Zhang, Hui-Zhong; Shen, Wan-An; Liu, Yan-Fang; Ma, Fu-Cheng (Department of Pathology, Xijing Hospital, Fourth Military Medical University, Xi'an, Shaanxi, 710033, Peop. Rep. China). World Journal of Gastroenterology, 9(3), 513-515 (English) 2003. CODEN: WJGAF2. ISSN: 1007-9327. Publisher: World Journal of Gastroenterology.

AB The RNase H gene of hepatitis B virus was amplified for the first half and second half (H1 and H2) by PCR from full length gene and cloned into pT7Blue-T vector. The ***RNase*** ***H1*** and H2 fragments isolated from XbaI and HindIII digestion products of pT7 Blue-RNase H plasmid were ligated to the GSTag expressing vectors sep., and expressed in E. coli BL21. The expressed proteins were checked by PAGE gel electrophoresis and Western blot. Expression of H1 and H2 fragments was confirmed by Western blot to be the GST and ***RNase*** ***H1***

or H2 fusion proteins.

L10 ANSWER 5 OF 22 CAPLUS COPYRIGHT 2003 ACS on STN
2003:298433 Document No. 139:34236 Transcriptional Gene Expression Profiling of Small Cell Lung Cancer Cells. Pedersen, Nina; Mortensen, Shila; Sorensen, Susanne B.; Pedersen, Mikkel W.; Rieneck, Klaus; Bovin, Lone F.; Poulsen, Hans Skovgaard (The Finsen Centre, Department of Radiation Biology, National University Hospital, Copenhagen, DK-2100, Den.). Cancer Research, 63(8), 1943-1953 (English) 2003. CODEN: CNREA8. ISSN: 0008-5472. Publisher: American Association for Cancer Research.
AB A global gene expression anal. using oligonucleotide microarrays was performed on many ***human*** small cell lung cancer (SCLC) cell lines in cell culture and/or as xenografts. The expression was compared with the expression profiles of 18 normal tissues. In a hierarchical cluster anal. the cell lines clustered distinctly from normal tissues and grouped into four clusters. One cluster consisted of two related cell lines and was markedly different from the other SCLC cell lines, whereas the rest of the clusters grouped together. Two subclusters contained the classical SCLC types and one subcluster the variant SCLC type, thus identifying many genes with differential expression between the two variants of SCLC. All of the xenografts clustered closest to the cell lines from which they originated and had the same expression levels as the cells grown in culture for the majority of genes. The anal. confirmed the high expression of many genes identified previously as highly expressed in SCLC cells including neuroendocrine markers, oncogenes, and genes involved in cell proliferation and division. The anal. furthermore identified a no. of mols. not identified previously as expressed in SCLC. Several of these are expressed in low or undetectable amts. in the majority of normal tissues and, therefore, are potential targets for new therapeutic approaches. By including the published array profiles of six ressected SCLC tumors from Bhattacharjee, J.; et al. (2001), the anal. revealed that most of the novel potential targets expressed by SCLC cell lines and xenografts were also expressed in the tumors. This anal. demonstrates the value of using cell lines and xenografts for expression profiling, when a limited quantity of tumor material is available.

L10 ANSWER 6 OF 22 CAPLUS COPYRIGHT 2003 ACS on STN
2003:96355 Document No. 138:148642 Method for the production of nucleic acids consisting of stochastically combined parts of source nucleic acids. Koltermann, Andre; Kettling, Ulrich; Greiner-Stoeffele, Thomas (Direvo Biotech AG, Germany). Eur. Pat. Appl. EP 1281757 A1 20030205, 23 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR. (English). CODEN: EPXXDW. APPLICATION: EP 2001-118416 20010731.
AB The invention relates to a method for the prodn. of nucleic acids consisting of stochastically combined parts of source nucleic acids as well as kits for carrying out synthesis. Methods for producing recombinant chimeric polynucleotides comprising: forming heteroduplexes with single-stranded, partially heterologous source nucleic acids which contain at least one marker nucleotide, introducing single-stranded nicks at sites of the marker nucleotides, and starting synthesis of single-stranded nucleic acids at the nick sites, the non-nicked strand serving as template.

L10 ANSWER 7 OF 22 CAPLUS COPYRIGHT 2003 ACS on STN
2003:18903 Document No. 138:249633 Excision of misincorporated ribonucleotides in DNA by RNase H (type 2) and FEN-1 in cell-free extracts. Rydberg, Bjorn; Game, John (Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA, 94720, USA). Proceedings of the National Academy of Sciences of the United States of America, 99(26), 16654-16659 (English) 2002. CODEN: PNASA6. ISSN: 0027-8424. Publisher: National Academy of Sciences.
AB Misincorporated ribonucleotides in DNA will cause DNA backbone distortion and may be targeted by DNA repair enzymes. Using double-stranded oligonucleotide probes contg. a single ribose, we demonstrate a robust activity in ***human*** , yeast, and Escherichia coli cell-free exts. that nicks 5' of the ribose. The ***human*** and yeast exts. also make a subsequent cut 3' of the ribonucleotide releasing a ribonucleotide monophosphate. The resulting 1-nt gap is an ideal substrate for polymerase and ligase to complete a proposed repair sequence that effectively replaces the ribose with deoxyribose. Screening of yeast

deletion mutant cells reveals that the initial nick is made by RNase H(35), a RNase H type 2 enzyme, and the second cut is made by Rad27p, the yeast homolog of ***human*** FEN-1 protein. RNase H type 2 enzymes are present in all kingdoms of life and are evolutionarily well conserved. We knocked out the corresponding rnhb gene in E. coli and show that exts. from this strain lack the nicking activity. Conversely, a highly purified archaeal RNase HII type 2 protein has a pronounced activity. To study substrate specificity, exts. were made from a yeast double mutant lacking the other main RNase H enzymes [***RNase*** ***H1*** and RNase H(70)], while maintaining RNase H(35). It was found that a single ribose is preferred as substrate over a stretch of riboses, further strengthening a proposed role of this enzyme in the repair of misincorporated ribonucleotides rather than (or in addn. to) processing RNA/DNA hybrid mols.

L10 ANSWER 8 OF 22 CAPLUS COPYRIGHT 2003 ACS on STN
2002:897472 Document No. 138:181786 Multiple ribonuclease H-encoding genes in the Caenorhabditis elegans genome contrasts with the two typical ribonuclease H-encoding genes in the ***human*** genome.
Arudchandran, Arulvathani; Cerritelli, Susana M.; Bowen, Nathan J.; Chen, Xiongfong; Krause, Michael W.; Crouch, Robert J. (Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, 20892, USA). Molecular Biology and Evolution, 19(11), 1910-1919 (English) 2002. CODEN: MBEVEO. ISSN: 0737-4038. Publisher: Society for Molecular Biology and Evolution.
AB Database searches of the Caenorhabditis elegans and ***human*** genomic DNA sequences revealed genes encoding ***RNase*** ***H1*** (***RNase*** ***H1***) and RNase H2 in each genome. The ***human*** genome contains a single copy of each gene, whereas C. elegans has four genes encoding ***RNase*** ***H1*** -related proteins and one gene for RNase H2. By analyzing the mRNAs produced from the C. elegans genes, examg. the amino acid sequence of the predicted protein, and expressing the proteins in Escherichia coli we have identified two active ***RNase*** ***H1*** -like proteins. One is similar to other eukaryotic ***RNases*** ***H1*** , whereas the second RNase H (rnh-1.1) is unique. The rnh-1.0 gene is transcribed as a dicistronic message with three dsRNA-binding domains; the mature mRNA is transspliced with SL2 splice leader and contains only one dsRNA-binding domain. Formation of ***RNase*** ***H1*** is further regulated by differential cis-splicing events. A single rnh-2 gene, encoding a protein similar to several other eukaryotic RNase H2L's, also has been examd. The diversity and enzymic properties of RNase H homologues are other examples of expansion of protein families in C. elegans. The presence of two ***RNases*** ***H1*** in C. elegans suggests that two enzymes are required in this rather simple organism to perform the functions that are accomplished by a single enzyme in more complex organisms. Phylogenetic anal. indicates that the active C. elegans ***RNases*** ***H1*** are distantly related to one another and that the C. elegans ***RNase*** ***H1*** is more closely related to the ***human*** ***RNase*** ***H1*** . The database searches also suggest that RNase H domains of LTR-retrotransposons in C. elegans are quite unrelated to cellular ***RNases*** ***H1*** , but numerous RNase H domains of ***human*** endogenous retroviruses are more closely related to cellular RNases H.

L10 ANSWER 9 OF 22 CAPLUS COPYRIGHT 2003 ACS on STN
2002:403305 Document No. 137:289641 Ribonuclease H1 maps to chromosome 2 and has at least three pseudogene loci in the ***human*** genome. ten Asbroek, Anneloor L. M. A.; van Groenigen, Marjon; Jakobs, Marja E.; Koevoets, Cindy; Janssen, Bert; Baas, Frank (Neurozintuigen Laboratory, Academic Medical Center, Amsterdam, 1000 DE, Neth.). Genomics, 79(6), 818-823 (English) 2002. CODEN: GNMCEP. ISSN: 0888-7543. Publisher: Elsevier Science.
AB We have analyzed the genomic structure of ***RNase*** ***H1*** (***RNase*** ***H1***) loci in the ***human*** genome. ***Human*** PAC library screening combined with database searches indicated that several loci are present. The transcribed gene is localized on chromosome 2p25. This was confirmed by RNA anal. of a monochromosomal hybrid cell line that expressed ***human*** chromosome 2. These data contradict a previous report, as well as the current ***Human*** Genome Project (HGP) annotation, which had placed the gene on chromosome 17p11.2. This location represents a pseudogene. Another

highly similar pseudogene is present at a sep. locus located more distal on chromosome 17p, while a third pseudogene is localized on chromosome 1q.

L10 ANSWER 10 OF 22 CAPLUS COPYRIGHT 2003 ACS on STN
2002:391845 Document No. 136:381396 Preparation of ***human*** ***RNase*** ***H1*** substitution and deletion mutants for determination of their domain functions. Wu, Hongjiang; Lima, Walter F.; Crooke, Stanley T. (Isis Pharmaceuticals, Inc., USA). PCT Int. Appl. WO 2002040635 A2 20020523, 39 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US43929 20011114. PRIORITY: US 2000-PV248950 20001115.

AB The present invention discloses the methods of prepn. of ***human*** ***RNase*** ***H1*** substitution and deletion mutants and assays for detn. of their domain functions. Specifically, the invention provides different deletion and substitution mutants, including putative catalytic site, basic substrate-binding domain, and some conserved amino acid residues. The functional anal. shows the domains that are responsible for ***RNase*** ***H1*** activity, and binding to the heteroduplex substrate.

L10 ANSWER 11 OF 22 CAPLUS COPYRIGHT 2003 ACS on STN
2002:108299 Document No. 136:395909 The involvement of ***human*** ribonucleases H1 and H2 in the variation of response of cells to antisense phosphorothioate oligonucleotides. ten Asbroek, Anneloor L. M. A.; Van Groenigen, Marjon; Nooij, Marleen; Baas, Frank (Neurozintuigen Laboratory, Academic Medical Center, Amsterdam, 1000 DE, Neth.). European Journal of Biochemistry, 269(2), 583-592 (English) 2002. CODEN: EJBCAI. ISSN: 0014-2956. Publisher: Blackwell Publishing Ltd..

AB The authors have analyzed the response of a no. of ***human*** cell lines to treatment with antisense oligodeoxynucleotides (ODNs) directed against RNA polymerase II, replication protein A, and Ha-ras. ODN-delivery to the cells was liposome-mediated or via electroporation, which resulted in different intracellular locations of the ODNs. The ODN-mediated target mRNA redn. varied considerably between the cell lines. In view of the essential role of RNase H activity in this response, RNase H was analyzed. The mRNA levels of ***RNase*** ***H1*** and RNase H2 varied considerably in the cell lines examd. in this study. The intracellular localization of the enzymes, assayed by green-fluorescent protein fusions, showed that ***RNase*** ***H1*** was present throughout the whole cell for all cell types analyzed, whereas RNase H2 was restricted to the nucleus in all cells except the prostate cancer line 15PC3 that expressed the protein throughout the cell. Whole cell exts. of the cell lines yielded similar RNase H cleavage activity in an in vitro liq. assay, in contrast to the efficacy of the ODNs in vivo. Overexpression of RNase H2 did not affect the response to ODNs in vivo. Our data imply that in vivo RNase H activity is not only due to the activity assayed in vitro, but also to an intrinsic property of the cells. ***RNase*** ***H1*** is not likely to be a major player in the antisense ODN-mediated degrdn. of target mRNAs. RNase H2 is involved in the activity assayed in vitro. The presence of cell-type specific factors affecting the activity and localization of RNase H2 is strongly suggested.

L10 ANSWER 12 OF 22 CAPLUS COPYRIGHT 2003 ACS on STN
2001:740505 Document No. 136:65112 Endogenous CRX expression and IRBP promoter activity in retinoblastoma cells. Boatright, J. H.; Borst, D. E.; Stodulkova, E.; Nickerson, J. M. (Emory Eye Center, Atlanta, GA, USA). Brain Research, 916(1,2), 136-142 (English) 2001. CODEN: BRREAP. ISSN: 0006-8993. Publisher: Elsevier Science B.V..

AB Purpose: To det. whether antisense oligonucleotides (AODNs) targeted against CRX, a photoreceptor-specific trans-acting factor, suppress CRX expression and interphotoreceptor retinoid binding protein (IRBP) promoter activity. Methods: Cultures of ***human*** retinoblastoma cells were transfected with chloramphenicol acetyltransferase (CAT) reporter plasmids contg. a mouse IRBP promoter and AODNs directed against CRX. RT-PCR using

primers specific to CRX, OTX2, GAPDH, or RNase H was conducted on total RNA isolated from retinoblastoma cells at various times following transfection with AODNs. Results: Transfection of retinoblastoma cells with IRBP promoter CAT constructs alone produced high activity. Co-transfection with AODNs suppressed IRBP promoter activity in a concn.-dependent manner, with half-maximal effect produced at about 2 nM AODN concn. Transfection with CAT constructs contg. an SV40 promoter produced high activity that was unaffected by co-transfection with AODNs. RT-PCR products were obtained for all target sequences. CRX RT-PCR product from cells transfected with AODNs was greatly diminished following transfection with an AODN whereas control transcripts, including that of OTX2, were relatively unaffected. Conclusions: The CRX-specific AODNs specifically and potently suppressed CRX expression and IRBP promoter activity, as measured by RT-PCR and transient transfection assays, resp. Little or no effect was seen on controls. These data suggest that endogenous CRX is required for IRBP promoter activity in retinoblastoma cells.

L10 ANSWER 13 OF 22 CAPLUS COPYRIGHT 2003 ACS on STN
2001:501049 Document No. 135:207389 Investigating the structure of ***human*** ***RNase*** ***H1*** by site-directed mutagenesis.
Wu, Hongjiang; Lima, Walt F.; Crooke, Stanley T. (Department of Molecular and Structural Biology, Isis Pharmaceuticals, Carlsbad, CA, 92008, USA). Journal of Biological Chemistry, 276(26), 23547-23553 (English) 2001. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB In this study we examine for the first time the roles of the various domains of ***human*** ***RNase*** ***H1*** by site-directed mutagenesis. The carboxyl terminus of ***human*** ***RNase*** ***H1*** is highly conserved with Escherichia coli ***RNase*** ***H1*** and contains the amino acid residues of the putative catalytic site and basic substrate-binding domain of the E. coli RNase enzyme. The amino terminus of ***human*** ***RNase*** ***H1*** contains a structure consistent with a double-strand RNA (dsRNA) binding motif that is sepd. from the conserved E. coli ***RNase*** ***H1*** region by a 62-amino acid sequence. These studies showed that although the conserved amino acid residues of the putative catalytic site and basic substrate-binding domain are required for RNase H activity, deletion of either the catalytic site or the basic substrate-binding domain did not ablate binding to the heteroduplex substrate. Deletion of the region between the dsRNA-binding domain and the conserved E. coli ***RNase*** ***H1*** domain resulted in a significant loss in the RNase H activity. Furthermore, the binding affinity of this deletion mutant for the heteroduplex substrate was .apprx.2-fold tighter than the wild-type enzyme suggesting that this central 62-amino acid region does not contribute to the binding affinity of the enzyme for the substrate. The dsRNA-binding domain was not required for RNase H activity, as the dsRNA-deletion mutants exhibited catalytic rates .apprx.2-fold faster than the rate obsd. for wild-type enzyme. Comparison of the dissocn. const. of ***human*** ***RNase*** ***H1*** and the dsRNA-deletion mutant for the heteroduplex substrate indicates that the deletion of this region resulted in a 5-fold loss in binding affinity. Finally, comparison of the cleavage patterns exhibited by the mutant proteins with the cleavage pattern for the wild-type enzyme indicates that the dsRNA-binding domain is responsible for the obsd. strong positional preference for cleavage exhibited by ***human*** ***RNase*** ***H1*** .

L10 ANSWER 14 OF 22 CAPLUS COPYRIGHT 2003 ACS on STN
2001:247542 Document No. 134:292059 ***Human*** RNase H and oligonucleotide compositions as substrates and for antisense therapy.
Crooke, Stanley T.; Lima, Walter F.; Wu, Hongjiang; Manoharan, Muthiah (Isis Pharmaceuticals, Inc., USA). PCT Int. Appl. WO 2001023613 A1 20010405, 178 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US26729 20000929. PRIORITY: US 1999-409926 19990930.

AB A ***human*** Type 2 RNase H has been cloned, expressed, and purified to electrophoretic homogeneity. The ***human*** RNase H is expressed ubiquitously in all tissues and cell lines tested except the MCR-5 line. The enzyme cleaves RNA in an oligonucleotide/RNA duplex, and the sites of cleavage in the full RNA/DNA substrate and in gapmer/RNA duplexes (in which the oligonucleotide gapmer has a 5-deoxynucleotide gap) were detd. The present invention provides oligonucleotides that can serve as substrates for ***human*** Type 2 RNase H and Escherichia coli ***RNase*** ***H1*** . These oligonucleotides are mixed sequence oligonucleotides comprising at least two portions, wherein a first portion is capable of supporting ***human*** ***RNase*** ***H1*** cleavage of a complementary target RNA and a further portions which is not capable of supporting such cleavage. To better characterize the substrate specificity of ***human*** RNase H, duplexes in which the antisense oligonucleotide is modified in the 2'-position were synthesized. The present invention is also directed to methods of using these oligonucleotides in enhancing antisense oligonucleotide therapies. Oligonucleotides can be screened to identify those which are effective antisense agents by contacting ***human*** RNase H with an oligonucleotide and measuring binding of the oligonucleotide to the enzyme. Antisense oligonucleotides are identified specific for the cleavage and inhibition of expression of ICAM-1, Ha-ras, c-raf, and 5-lipoxygenase messages.

L10 ANSWER 15 OF 22 CAPLUS COPYRIGHT 2003 ACS on STN
1999:651357 Document No. 131:348275 Properties of cloned and expressed ***human*** ***RNase*** ***H1*** . Wu, Hongjiang; Lima, Walt F.; Crooke, Stanley T. (Isis Pharmaceuticals, Inc., Carlsbad, CA, 92082, USA). Journal of Biological Chemistry, 274(40), 28270-28278 (English) 1999. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB We have characterized cloned His-tag ***human*** ***RNase*** ***H1*** . The activity of the enzyme exhibited a bell-shaped response to divalent cations and pH. The optimum conditions for catalysis consisted of 1 mM Mg2+ and pH 7-8. In the presence of Mg2+, Mn2+ was inhibitory. ***Human*** ***RNase*** ***H1*** shares many enzymic properties with Escherichia coli ***RNase*** ***H1*** . The ***human*** enzyme cleaves RNA in a DNA-RNA duplex resulting in products with 5'-phosphate and 3'-hydroxy termini, can cleave overhanging single strand RNA adjacent to a DNA-RNA duplex, and is unable to cleave substrates in which either the RNA or DNA strand has 2' modifications at the cleavage site. ***Human*** ***RNase*** ***H1*** binds selectively to "A-form"-type duplexes with approx. 10-20-fold greater affinity than that obsd. for E. coli ***RNase*** ***H1*** . The ***human*** enzyme displays a greater initial rate of cleavage of a heteroduplex-contg. RNA-phosphorothioate DNA than an RNA-DNA duplex. Unlike the E. coli enzyme, ***human*** ***RNase*** ***H1*** displays a strong positional preference for cleavage, i.e., it cleaves between 8 and 12 nucleotides from the 5'-RNA-3'-DNA terminus of the duplex. Within the preferred cleavage site, the enzyme displays modest sequence preference with GU being a preferred dinucleotide. The enzyme is inhibited by single-strand phosphorothioate oligonucleotides and displays no evidence of processivity. The min. RNA-DNA duplex length that supports cleavage is 6 base pairs, and the min. RNA-DNA "gap size" that supports cleavage is 5 base pairs.

L10 ANSWER 16 OF 22 CAPLUS COPYRIGHT 2003 ACS on STN
1998:762584 Document No. 130:134758 Cloning, expression and mapping of ribonucleases H of ***human*** and mouse related to bacterial RNase HI. Cerritelli, Susana M.; Crouch, Robert J. (Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, 20892, USA). Genomics, 53(3), 300-307 (English) 1998. CODEN: GNMCEP. ISSN: 0888-7543. Publisher: Academic Press.

AB We identified two ***human*** sequences and one mouse sequence in the database of expressed sequence tags that are highly homologous to the N-terminal sequence of eukaryotic ***RNases*** ***H1*** . The cDNAs for ***human*** RNASEH1 and mouse Ranaseh1 were obtained, their nucleotide sequences detd., and the proteins expressed in Escherichia coli and partially purified. Both proteins have RNase H activity in vitro and they bind to dsRNA and RNA-DNA hybrids through the N-terminal conserved

motif present in eukaryotic ***RNases*** ***H1*** . The RNASEH1 gene is expressed in all ***human*** tissues at similar levels, indicating that ***RNase*** ***H1*** may be a housekeeping protein. The ***human*** RNASEH1 and mouse Rnaseh1 cDNAs were used to isolate BAC genomic clones that were used as probes for fluorescence in situ hybridization. The ***human*** gene was localized to chromosome 17p11.2 and the mouse gene to a nonsyntenic region on chromosome 12A3. The chromosomal location and possible disease assocn. of the ***human*** RNASEH1 gene are discussed. (c) 1998 Academic Press.

L10 ANSWER 17 OF 22 CAPLUS COPYRIGHT 2003 ACS on STN
1998:296363 Document No. 129:64623 Molecular mechanisms of antisense drugs: RNase H. Crooke, Stanley T. (Isis Pharmaceuticals, Inc., Carlsbad, CA, 92008, USA). Antisense & Nucleic Acid Drug Development, 8(2), 133-134 (English) 1998. CODEN: ANADF5. ISSN: 1087-2906. Publisher: Mary Ann Liebert, Inc..
AB A review with 9 refs. on the studies conducted in the lab. of Stanley T. Crooke on Escherichia coli ***RNase*** ***H1*** and ***human*** RNase H type 2. Although a no. of mechanisms by which antisense drugs induce their effects have been identified, RNase H-mediated degrdn. of targeted RNA is perhaps the best documented. To better understand how antisense drugs that form duplexes with RNA that serve as substrates for RNase H work, the authors have conducted studies on Escherichia coli ***RNase*** ***H1*** and ***human*** RNase H type 2. The topics in the review include: structure-activity relationships of antisense drugs interacting with E. coli RNase H; effects of RNA structure on activity of E. coli RNase H and the cloning and expression of ***human*** type 2 RNase H. Insights into the characteristics of RNase H will provide crucial direction for future work in medicinal chem. and pharmacol. The cloning and expression of ***human*** RNase H enzymes should provide addnl. support for improved antisense therapeutics.

L10 ANSWER 18 OF 22 CAPLUS COPYRIGHT 2003 ACS on STN
1995:479833 Document No. 123:50966 Substitution of a highly basic helix/loop sequence into the RNase H domain of ***human*** immunodeficiency virus reverse transcriptase restores its Mn2+-dependent RNase H activity. Keck, James L.; Marqusee, Susan (Department of Molecular and Cell Biology, University of California, Berkeley, CA, 94720, USA). Proceedings of the National Academy of Sciences of the United States of America, 92(7), 2740-4 (English) 1995. CODEN: PNASA6. ISSN: 0027-8424. Publisher: National Academy of Sciences.
AB ***Human*** immunodeficiency virus (HIV) reverse transcriptase (RT) is a multifunctional protein, contg. both DNA polymerase and RNase H activity. The RNase H activity of HIV RT catalyzes the hydrolysis of the RNA strand of RNA.cntdot.DNA hybrids. Although the domain that carries out the RNase H activity in HIV RT can be expressed as an independent, folded polypeptide, it is inactive as an RNase H. This report describes the overexpression and purifn. of an active, recombinant HIV RNase H domain in which the sequence corresponding to the Escherichia coli ***RNase*** ***H1*** basic helix/loop was substituted for the corresponding sequence of HIV RNase H. The resulting polypeptide (RNH102) has Mn2+-dependent RNase H activity and is more stable than the independently expressed wild-type HIV RNase H domain.

L10 ANSWER 19 OF 22 CAPLUS COPYRIGHT 2003 ACS on STN
1993:508374 Document No. 119:108374 Phosphorothioate oligonucleotides are inhibitors of ***human*** DNA polymerases and RNase H: Implications for antisense technology. Gao, Wen Yi; Han, Fu Sheng; Storm, Christy; Egan, William; Cheng, Yung Chi (Sch. Med., Yale Univ., New Haven, CT, 06510, USA). Molecular Pharmacology, 41(2), 223-9 (English) 1992. CODEN: MOPMA3. ISSN: 0026-895X.
AB Phosphorothioate oligodeoxycytidine (S-dCn) was used as a model compd. to examine the impact of the no. of phosphorothioate linkages and their position on the inhibition of ***human*** DNA polymerases and RNase H in vitro. S-dCn with a chain length >15 could inhibit ***human*** DNA polymerases and RNase H activities in a linkage no.-dependent manner. Longer oligomers were more potent inhibitors than shorter ones. Kinetic studies indicated that S-dC28 was a competitive inhibitor of DNA polymerase .alpha. and .beta. with respect to the DNA template, whereas it was a noncompetitive inhibitor of polymerases .gamma. and .delta.. S-dC28 was also a competitive inhibitor of ***RNase*** ***H1*** and H2

with respect to RNA-DNA duplex. Susceptibility of these enzymes to inhibition by S-dC28 was in the order of .delta. .apprxeq. .gamma. > .alpha. > .beta. and ***RNase*** ***H1*** > RNase H2. Structural-activity relations were explored with a group of S-dC28 analogs that have phosphorothioate internucleotide linkages at various positions. The inhibitory effect depended on the total no. of thioate linkages, rather than the position of the linkages within the oligomer or the chain length itself. No sequence specificity was found. In the presence of the complementary RNA, antisense phosphorothioates (S-oligos) exerted a biphasic effect on RNase H activity. At low concns. S-oligos could enhance the cleavage of the RNA portion of S-oligo-RNA duplex, whereas at high concns. (in excess of the complementary RNA) S-oligos could inhibit RNase H and protect the complementary RNA from degrdn. Together, these results suggest that the nonsequence-specific inhibitory effect of S-oligos should be taken into consideration in designing antisense inhibitors. This inhibitory activity could be avoided by decreasing the no. of phosphorothioate linkages at the backbone, and S-oligos of 15-20 residues are preferable in antisense mol. design.

L10 ANSWER 20 OF 22 CAPLUS COPYRIGHT 2003 ACS on STN
1993:423448 Document No. 119:23448 Substrate specificity of ***human*** ***RNase*** ***H1*** and its role in excision repair of ribose residues misincorporated in DNA. Eder, P. S.; Walder, R. Y.; Walder, J. A. (Dep. Biochem., Univ. Iowa, Iowa City, IA, 52242, USA). Biochimie, 75(1-2), 123-6 (English) 1993. CODEN: BICMBE. ISSN: 0300-9084.

AB The major isoform of RNase H in ***human*** cells, ***RNase*** ***H1*** , is able to cleave DNA substrates contg. a single RNA-DNA base pair, an activity which appears to be involved in an excision repair system for the removal of ribose residues misincorporated into DNA. In the present work, the substrate specificity of the enzyme was further characterized. DNA substrates contg. all four ribonucleotides are cleaved by the enzyme. A RNA-DNA base pair is not required for substrate recognition; RNA residues present within a mismatch or a RNA-RNA base pair are also cleaved. The principal structural feature for recognition by the enzyme may simply be the presence of the 2'-OH group of the ribose residue adjacent to the cleavage site.

L10 ANSWER 21 OF 22 CAPLUS COPYRIGHT 2003 ACS on STN
1992:527311 Document No. 117:127311 The effects of cysteine mutations on the catalytic activities of the reverse transcriptase of ***human*** immunodeficiency virus type-1. Loya, Shoshana; Tal, Ruth; Hughes, Stephen H.; Hizi, Amnon (Sackler Sch. Med., Tel Aviv Univ., Tel Aviv, 69978, Israel). Journal of Biological Chemistry, 267(20), 13879-83 (English) 1992. CODEN: JBCHA3. ISSN: 0021-9258.

AB The reverse transcriptase (RT) of the ***human*** immunodeficiency virus type 1 (HIV-1) has only 2 cysteine residues at positions 38 and 280. In order to investigate the role of these cysteines in the structure and function of the enzyme, each of the cysteines was previously modified to serines employing site-directed mutagenesis. Two of the mutant forms of HIV-1 RT, the single mutant of cysteine 280 and a double mutant with both cysteines modified, were purified. In the present study, the catalytic properties of the DNA-polymg. and the RNase H functions of the two mutant RTs were compared to those of the native enzyme. The results indicate that the single mutant RT closely resembles the wild-type enzyme in almost all the catalytic functions tested. The double cysteine mutant RT, on the other hand, exhibits several unique features. First, the specific activities of the RNA- and DNA-directed DNA synthesis are significantly lower than the corresponding activities of the other two enzymes. This probably results from the lower Vmax values exhibited by the double mutant RT, since the Km values calcd. for all enzymes were similar. Second, the most outstanding differences are assocd. with the RNase H activity of the double mutant RT. The specific activity of RNase H is about 4-fold higher than the wild type and the single mutant RTs. Furthermore, the heat stability of the RNase H function of the double-mutated RT is at least 15-fold higher than that of the other two RTs. The substantial resistance to heat denaturation is apparent only for the RNase H activity, since the DNA polymg. function of the double mutant RT is as sensitive to heat denaturation as the other two proteins.

L10 ANSWER 22 OF 22 CAPLUS COPYRIGHT 2003 ACS on STN
1991:601694 Document No. 115:201694 Ribonuclease H from K562 ***human***

erythroleukemia cells. Purification, characterization, and substrate specificity. Eder, Paul S.; Walder, Joseph A. (Dep. Biochem., Univ. Iowa, Iowa City, IA, 52242, USA). Journal of Biological Chemistry, 266(10), 6472-9 (English) 1991. CODEN: JBCHA3. ISSN: 0021-9258.

AB The major RNase H from K562 ***human*** erythroleukemia cells was purified >4000-fold. This RNase H, now termed ***RNase*** ***H1*** , is an endoribonuclease whose products contain 5'-phosphoryl and 3'-hydroxyl termini. The enzyme had a native mol. wt. of 89,000 based on its sedimentation and diffusion coeffs. ***Human*** ***RNase*** ***H1*** had an abs. requirement for a divalent cation. Maximal activity was obtained with either 10 mM Mg2+, 5 mM Co2+, or 0.5 mM Mn2+. The pH optimum was 8.0-8.5 in the presence of 10 mM Mg2+. The pI was 6.4. ***RNase*** ***H1*** lacked double-stranded and single-stranded RNase and DNase activities, and it would not hydrolyze the DNA moiety of an RNA.cntdot.DNA heteroduplex. Unlike the Escherichia coli enzyme, which requires a heteroduplex that contains at least 4 consecutive ribonucleotides for activity, ***human*** ***RNase*** ***H1*** could hydrolyze a DNA.cntdot.RNA.cntdot.DNA/DNA heteroduplex that contains a single ribonucleotide. Cleavage occurred at the 5' phosphodiester of this residue. This substrate specificity suggested that ***human*** ***RNase*** ***H1*** could play a role in ribonucleotide excision from genomic DNA during replication.

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=> S L9 AND MUTATION?
        244428 MUTATION?
L11        115 L9 AND MUTATION?

L12        115 L9 (6A) MUTATION?

=> E WU H/AU
=> S E3
L13        236 "WU H"/AU

=> E WU HONG/AU
=> S E19
L14          3 "WU HONG J"/AU

=> E WU HONGJANG/AU
=> S E3
L15          1 "WU HONGJANG"/AU

=> E LIMA W/AU
=> S E3,E13
             1 "LIMA W"/AU
             5 "LIMA WALTER"/AU
L16          6 ("LIMA W"/AU OR "LIMA WALTER"/AU)

=> E CROOKE S/AU
=> S E3-E10
             1 "CROOKE S"/AU
            27 "CROOKE S T"/AU
             2 "CROOKE STANELY T"/AU
             1 "CROOKE STANEY"/AU
             2 "CROOKE STANLEY"/AU
             1 "CROOKE STANLEY I"/AU
           536 "CROOKE STANLEY T"/AU
             1 "CROOKE STANLY T"/AU
L17        571 ("CROOKE S"/AU OR "CROOKE S T"/AU OR "CROOKE STANELY T"/AU OR
               "CROOKE STANEY"/AU OR "CROOKE STANLEY"/AU OR "CROOKE STANLEY
               I"/AU OR "CROOKE STANLEY T"/AU OR "CROOKE STANLY T"/AU)

=> S L13,L14,L15,L16,L17
L18        815 (L13 OR L14 OR L15 OR L16 OR L17)

=> S L18 AND (L3,L4)
L19         21 L18 AND ((L3 OR L4))

=> S L19 NOT L10
L20         15 L19 NOT L10
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=> D 1-15 CBIB ABS

L20 ANSWER 1 OF 15 CAPLUS COPYRIGHT 2003 ACS on STN
2003:590873 Document No. 139:129918 Protein and nucleotide sequences of human ***RNase*** ***H*** and uses thereof. ***Crooke, Stanley*** *** T.*** ; ***Lima, Walter*** ; Wu, Hongjiang (USA). U.S. Pat. Appl. Publ. US 2003144496 A1 20030731, 14 pp., Cont.-in-part of U.S. Ser. No. 861,205. (English). CODEN: USXXCO. APPLICATION: US 2003-358439 20030203. PRIORITY: US 1997-PV67458 19971204; US 1998-203716 19981202; US 1999-343809 19990630; US 2000-684254 20001006; US 2001-861205 20010518.
AB The present invention provides polynucleotides and polypeptides encoded thereby of human Type 2 ***RNase*** ***H*** . The invention relates to methods of using these polynucleotides and polypeptides in enhancing antisense oligonucleotide therapies are also provided.

L20 ANSWER 2 OF 15 CAPLUS COPYRIGHT 2003 ACS on STN
2003:146118 Document No. 139:127954 Efficient Reduction of Target RNAs by Small Interfering RNA and ***RNase*** ***H*** -dependent Antisense Agents. A comparative analysis. Vickers, Timothy A.; Koo, Seongjoon; Bennett, C. Frank; ***Crooke, Stanley T.*** ; Dean, Nicholas M.; Baker, Brenda F. (GeneTrove Division and Antisense Core Research Department, Isis Pharmaceuticals, Inc., Carlsbad, CA, 92008, USA). Journal of Biological Chemistry, 278(9), 7108-7118 (English) 2003. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.
AB RNA interference can be considered as an antisense mechanism of action that utilizes a double-stranded RNase to promote hydrolysis of the target RNA. We have performed a comparative study of optimized antisense oligonucleotides designed to work by an RNA interference mechanism to oligonucleotides designed to work by an ***RNase*** ***H*** -dependent mechanism in human cells. The potency, maximal effectiveness, duration of action, and sequence specificity of optimized ***RNase*** ***H*** -dependent oligonucleotides and small interfering RNA (siRNA) oligonucleotide duplexes were evaluated and found to be comparable. Effects of base mismatches on activity were detd. to be position-dependent for both siRNA oligonucleotides and ***RNase*** ***H*** -dependent oligonucleotides. In addn., we detd. that the activity of both siRNA oligonucleotides and ***RNase*** ***H*** -dependent oligonucleotides is affected by the secondary structure of the target mRNA. To det. whether positions on target RNA identified as being susceptible for ***RNase*** ***H*** -mediated degrdn. would be coincident with siRNA target sites, we evaluated the effectiveness of siRNAs designed to bind the same position on the target mRNA as ***RNase*** ***H*** -dependent oligonucleotides. Examn. of 80 siRNA oligonucleotide duplexes designed to bind to RNA from four distinct human genes revealed that, in general, activity correlated with the activity to ***RNase*** ***H*** -dependent oligonucleotides designed to the same site, although some exceptions were noted. The one major difference between the two strategies is that ***RNase*** ***H*** -dependent oligonucleotides were detd. to be active when directed against targets in the pre-mRNA, whereas siRNAs were not. These results demonstrate that siRNA oligonucleotide- and ***RNase*** ***H*** -dependent antisense strategies are both valid strategies for evaluating function of genes in cell-based assays.

L20 ANSWER 3 OF 15 CAPLUS COPYRIGHT 2003 ACS on STN
2002:356125 Document No. 137:105527 Human ***RNases*** ***H*** . Lima, Walt F.; Wu, Hongjiang; ***Crooke, Stanley T.*** (Department of Molecular and Structural Biology, Isis Pharmaceuticals Inc., Carlsbad, CA, 92008, USA). Methods in Enzymology, 341(Ribonucleases, Part A), 430-440 (English) 2001. CODEN: MENZAU. ISSN: 0076-6879. Publisher: Academic Press.
AB A review. (c) 2001 Academic Press.

L20 ANSWER 4 OF 15 CAPLUS COPYRIGHT 2003 ACS on STN
2002:17629 Document No. 137:103197 Phosphorodiamidate morpholino oligomers. Iversen, Patrick (AVI BioPharma, Inc., Corvallis, OR, USA). Antisense Drug Technology, 375-389. Editor(s): ***Crooke, Stanley T*** . Marcel Dekker, Inc.: New York, N. Y. ISBN: 0-8247-0566-1 (English) 2001. CODEN: 69CEBQ.
AB A review on phosphorodiamidate morpholino oligomers (PMOs), which

represent a non-ionic and non- ***RNase*** ***H*** -mediated approach to antisense inhbition of gene expression. The mechanisms of action involve both interference with ribosomal assembly, thus preventing translation, and interference with intron-exon splicing of pre-mRNA preventing appropriate translation of selected mRNA. PMOs have showed in vivo efficacy in multiple animal models of cancer, polycystic kidney disease, liver regeneration, and vascular restenosis following balloon injury assocd. with angioplasty. In vivo efficacy has been obsd. in mouse, rat, rabbit, and pig models. The in vivo efficacy of PMOs has involved both single-dose administration and multiple daily doses for up to 14 days. The efficacy of PMOs has also been obsd. following i.v., i.p., s.c., transdermal, and oral administration.

L20 ANSWER 5 OF 15 CAPLUS COPYRIGHT 2003 ACS on STN
2001:851765 Document No. 135:376714 Methods of using mammalian ***RNase*** ***H*** and compositions thereof in antisense technology. Monia, Brett P.; Cook, Phillip Dan; ***Crooke, Stanley T.*** ; ***Lima, Walter*** ; Wu, Hongjiang (USA). U.S. Pat. Appl. Publ. US 20010044145 A1 20011122, 30 pp., Cont.-in-part of U.S. Ser. No. 781,712. (English). CODEN: USXXCO. APPLICATION: US 2001-799848 20010305. PRIORITY: US 1991-814961 19911224; US 1994-244993 19940621; US 1997-861306 19970421; US 1997-PV67458 19971204; WO 1998-US13966 19980706; US 1998-144611 19980831; US 1998-203716 19981202; US 1999-343809 19990630; US 1999-453514 19991201; US 2000-462280 20000301; US 2000-684254 20001006; US 2001-781712 20010212.
AB The present invention relates to methods for using mammalian ***RNase*** ***H*** and compns. thereof, particularly for redn. of a selected cellular RNA target via antisense technol.

L20 ANSWER 6 OF 15 CAPLUS COPYRIGHT 2003 ACS on STN
2001:48032 Document No. 134:305259 Variations in mRNA content have no effect on the potency of antisense oligonucleotides. Miraglia, Loren; Watt, Andrew T.; Graham, Mark J.; ***Crooke, Stanley T.*** (Isis Pharmaceuticals, Inc., Carlsbad, CA, 92008, USA). Antisense & Nucleic Acid Drug Development, 10(6), 453-461 (English) 2000. CODEN: ANADF5. ISSN: 1087-2906. Publisher: Mary Ann Liebert, Inc..
AB A fundamental question with regard to antisense pharmacol. is the extent to which RNA content or transcription rate or both affect the potency of antisense drugs. We have addressed this by controlling RNA content and transcription rate using either an exogenous gene expressed after transfection or an endogenous gene induced with a cytokine. We have demonstrated that in both A549 and HeLa cells, varying RNA copy nos. from <1 to >100 copies per cell has no effect on the potency of ***RNase*** ***H*** -active antisense drugs transfected into cells, nor did variation in transcription rate have an effect on potency. We demonstrate that this is because the no. of oligonucleotide mols. per cell is vastly in excess of the RNA copy no. These data further suggest that a significant fraction of cell-assocd. antisense drug mols. may be unavailable to interact with the target RNA, an observation that is not surprising, as phosphorothioate oligonucleotides interact with many cellular proteins. We suggest that these data may extrapolate to in vivo results.

L20 ANSWER 7 OF 15 CAPLUS COPYRIGHT 2003 ACS on STN
1999:760671 Document No. 132:160698 Molecular mechanisms of action of antisense drugs. ***Crooke, S. T.*** (Isis Pharmaceuticals, Inc., Carlsbad, CA, USA). Biochimica et Biophysica Acta, 1489(1), 31-43 (English) 1999. CODEN: BBACAQ. ISSN: 0006-3002. Publisher: Elsevier Science B.V..
AB A review with 106 refs. Given the progress reported during the past decade, a wide range of chem. modifications may be incorporated into potential antisense drugs. These modifications may influence all the properties of these mols., including mechanism of action. DNA-like antisense drugs have been shown to serve as substrates when bound to target RNAs for RNase Hs. These enzymes cleave the RNA in RNA/DNA duplexes and now the human enzymes have been cloned and characterized. A no. of mechanisms other than ***RNase*** ***H*** have also been reported for non-DNA-like antisense drugs. For example, activation of splicing, inhibition of 5'-cap formation, translation arrest and activation of double strand RNases have all been shown to be potential mechanisms. Thus, there is a growing repertoire of potential mechanisms of action from which to choose, and a range of modified oligonucleotides to match to the desired mechanism. Further, we are beginning to

understand the various mechanisms in more detail. These insights, coupled with the ability to rapidly evaluate activities of antisense drugs under well-controlled rapid throughput systems, suggest that we will make more rapid progress in identifying new mechanisms, developing detailed understanding of each mechanism and creating oligonucleotides that better predict what sites in an RNA are most amenable to antisense drugs of various chem. classes.

L20 ANSWER 8 OF 15 CAPLUS COPYRIGHT 2003 ACS on STN
1999:548317 Document No. 131:280844 Molecular mechanisms of antisense drugs: human ***RNase*** ***H*** . ***Crooke, Stanley T.*** (Isis Pharmaceuticals, Inc., Carlsbad, CA, 92008, USA). Antisense & Nucleic Acid Drug Development, 9(4), 377-379 (English) 1999. CODEN: ANADF5. ISSN: 1087-2906. Publisher: Mary Ann Liebert, Inc..
AB A review with 29 refs. Activation of ***RNase*** ***H*** enzymes is a key mechanism of action of DNA-like antisense drugs. With the cloning and expression of the human enzymes, we now have the tools to prove that ***RNase*** ***H*** are involved in antisense effects and to design drugs that take better advantage of this mechanism.

L20 ANSWER 9 OF 15 CAPLUS COPYRIGHT 2003 ACS on STN
1999:375648 Document No. 131:29292 Human ***RNase*** ***H*** and compositions and uses in screening and production of antisense oligonucleotides. ***Crooke, Stanley T.*** ; Lima, Walter F.; Wu, Hongjiang (Isis Pharmaceuticals, Inc., USA). PCT Int. Appl. WO 9928447 A1 19990610, 37 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1998-US25488 19981202. PRIORITY: US 1997-67458 19971204.
AB The present invention relates to a human type 2 ***RNase*** ***H*** which has been cloned, expressed, and purified to electrophoretic homogeneity and to the use human ***RNase*** ***H*** and various compns. for assays and therapies. The human ***RNase*** ***H*** is highly homologous to Escherichia coli RNase HI, its cleavage characteristics are consistent with a type 2 enzyme, and it is expressed ubiquitously in human cells and tissues. Methods of using these polynucleotides and polypeptides in enhancing antisense oligonucleotide therapies are provided. Methods of screening for effective antisense oligonucleotides and of producing effective antisense oligonucleotides using human type 2 ***RNase*** ***H*** are also provided.

L20 ANSWER 10 OF 15 CAPLUS COPYRIGHT 2003 ACS on STN
1998:158638 Document No. 128:292091 Molecular cloning and expression of cDNA for human ***RNase*** ***H*** . Wu, Hongjiang; Lima, Walt F.; ***Crooke, Stanley T.*** (Department of Molecular Pharmacology, Isis Pharmaceuticals, Carlsbad, CA, 92008, USA). Antisense & Nucleic Acid Drug Development, 8(1), 53-61 (English) 1998. CODEN: ANADF5. ISSN: 1087-2906. Publisher: Mary Ann Liebert, Inc..
AB We have cloned, expressed, and purified to electrophoretic homogeneity a human ***RNase*** ***H*** . The enzyme has a mol. wt. of 32 kDa, is Mg2+ dependent, and is inhibited by Mn2+ and N-ethylmaleimide. Its mol. wt. and cleavage characteristics are consistent with type 2 human ***RNase*** ***H*** . The human ***RNase*** ***H*** we have cloned is highly homologous to Escherichia coli RNase HI (33.6% amino acid identity) and to other ***RNase*** ***H*** enzymes homologous to E. coli RNase HI. The enzyme is encoded by a single gene that is at least 10 kb in length and is expressed ubiquitously in human cells and tissues.

L20 ANSWER 11 OF 15 CAPLUS COPYRIGHT 2003 ACS on STN
1997:729584 Document No. 128:58890 Cleavage of single strand RNA adjacent to RNA-DNA duplex regions by Escherichia coli ***RNase*** ***H1*** . Lima, Walt F.; ***Crooke, Stanley T.*** (Isis Pharmaceuticals, Inc., Carlsbad, CA, 92008, USA). Journal of Biological Chemistry, 272(44), 27513-27516 (English) 1997. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.
AB ***RNase*** ***H1*** from Escherichia coli cleaves single strand

RNA extending 3' from an RNA-DNA duplex. Substrates consisting of a 25-mer RNA annealed to complementary DNA ranging in length from 9-17 nucleotides were designed to create overhanging single strand RNA regions extending 5' and 3' from the RNA-DNA duplex. Digestion of single strand RNA was obsd. exclusively within the 3' overhang region and not the 5' overhang region. ***RNase*** ***H*** digestion of the 3' overhang region resulted in digestion products with 5'-phosphate and 3'-hydroxyl termini. The no. of single strand RNA residues cleaved by ***RNase*** ***H*** is influenced by the sequence of the single strand RNA immediately adjacent to the RNA-DNA duplex and appears to be a function of the stacking properties of the RNA residues adjacent to the RNA-DNA duplex. ***RNase*** ***H*** digestion of the 3' overhang region was not obsd. for a substrate that contained a 2'-methoxy antisense strand. The introduction of 3 deoxynucleotides at the 5' terminus of the 2'-methoxy antisense oligonucleotide resulted in cleavage. These results offer addnl. insights into the binding directionality of ***RNase*** ***H*** with respect to the heteroduplex substrate.

L20 ANSWER 12 OF 15 CAPLUS COPYRIGHT 2003 ACS on STN
1997:476853 Document No. 127:216855 The influence of antisense oligonucleotide-induced RNA structure on Escherichia coli ***RNase*** ***H1*** activity. Lima, Walt F.; Mohan, Venkatraman; ***Crooke,*** ***Stanley T.*** (Isis Pharm., Inc., Carlsbad, CA, 92008, USA). Journal of Biological Chemistry, 272(29), 18191-18199 (English) 1997. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB The ability of Escherichia coli ***RNase*** ***H1*** to hydrolyze structured substrates contg. antisense oligonucleotides preannealed to a 47-mer RNA was compared with its ability to hydrolyze unstructured substrates contg. antisense oligonucleotides duplexed with 13-mer RNA. These results demonstrate that when antisense oligonucleotides were bound to structured RNA, the resultant duplexes were cleaved at rates significantly slower than when the same oligonucleotides were bound to unstructured oligoribonucleotides. Structured substrates exhibited fewer cleavage sites, and each cleavage site was cleaved less rapidly than in unstructured substrates. Furthermore, the enzymic activity of E. coli ***RNase*** ***H1*** for the structured substrates was most affected when the cleavage sites corresponding to the enzymically most active sites on the unstructured substrates were blocked in the structured substrates. Mol. modeling suggests that the obsd. ablation of ***RNase*** ***H*** activity was due to the steric hindrance of the enzyme by the structured RNA, i.e. steric interference of the phosphate groups on the substrate and/or the binding site of the enzyme. When chimeric oligonucleotides composed of a five-base deoxynucleotide sequence flanked by chem. modified nucleotides were bound to structured RNA, the resultant duplexes were even worse substrates for ***RNase*** ***H*** . These results offer further insights into the role of antisense-induced RNA structure on ***RNase*** ***H*** activity and may facilitate the design of effective antisense oligonucleotides.

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1997:96770 Document No. 126:86393 Binding Affinity and Specificity of Escherichia coli ***RNase*** ***H1*** : Impact on the Kinetics of Catalysis of Antisense Oligonucleotide-RNA Hybrids. Lima, Walt F.; ***Crooke, Stanley T.*** (Isis Pharmaceuticals Inc., Karlovy vary, CA, 92008, USA). Biochemistry, 36(2), 390-398 (English) 1997. CODEN: BICHAW. ISSN: 0006-2960. Publisher: American Chemical Society.

AB In this study we report for the first time the binding affinity of ***RNase*** ***H1*** for oligonucleotide duplexes. We used a previously described 17-mer antisense sequence [Monia, B. P., Johnston, J. F., Ecker, D. J., Zounes, M. A., Lima, W. F., & Freier, S. M. (1992) J. Biol. Chem. 267, 19954-19962] hybridized to a complementary oligoribonucleotide to evaluate both the binding affinity and the catalytic rate of ***RNase*** ***H1*** . The dissocn. consts. (Kd) of ***RNase*** ***H1*** for the various substrates tested were detd. by inhibition anal. using chem. modified noncleavable oligonucleotide heteroduplexes. Catalytic rates were detd. using heteroduplex substrates contg. chimeric antisense oligonucleotides composed of a five-base deoxynucleotide sequence flanked on either side by chem. modified nucleotides. We find that the enzyme preferentially binds A-form duplexes: ***RNase*** ***H*** bound A-form duplexes

(RNA:RNA and DNA:RNA) approx. 60-fold tighter than B-form duplexes (DNA:DNA) and approx. 300-fold tighter than single-strand oligonucleotides. The enzyme exhibited equal affinity for both the wild type (RNA:DNA) oligonucleotide substrate and heteroduplexes contg. various 2'-sugar modifications, while the cleavage rates for these chem. modified substrates were without exception slower than for the wild type substrate. The introduction of a single pos. charged 2'-propoxyamine modification into the chimeric antisense oligonucleotide portion of the heteroduplex substrate resulted in both decreased binding affinity and a slower rate of catalysis by ***RNase*** ***H*** . The cleavage rates for heteroduplexes contg. single-base mismatch sequences within the chimeric oligonucleotide portion varied depending on the position of the mismatch but had no effect on the binding affinity of the enzyme. These results offer further insights into the phys. binding properties of the ***RNase*** ***H*** -substrate interaction as well as the design of effective antisense oligonucleotides.

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1995:983437 Document No. 124:49156 Kinetic characteristics of Escherichia coli ***RNase*** ***H1*** : cleavage of various antisense oligonucleotide-RNA duplexes. ***Crooke, Stanley T.*** ; Lemonidis, Kristina M.; Neilson, Lorna; Griffey, Richard; Lesnik, Elena A.; Monia, Brett P. (Isis Pharmaceuticals, Inc., Karlovy vary, CA, 92008, USA). Biochemical Journal, 312(2), 599-608 (English) 1995. CODEN: BIJOAK. ISSN: 0264-6021. Publisher: Portland Press.

AB The effects of variations in substrates on the kinetic properties of Escherichia coli ***RNase*** ***H*** were studied using antisense oligonucleotides of various types hybridized to complementary oligoribonucleotides. The enzyme displayed minimal sequence preference, initiated cleavage through an endonucleolytic mechanism near the 3' terminus of the RNA in a DNA-RNA chimera and then was processively exonucleolytic. Phosphorothioate oligodeoxynucleotides hybridized to RNA supported cleavage more effectively than phosphodiester oligodeoxynucleotides. Oligonucleotides comprised of 2'-methoxy-, 2'-fluoro- or 2'-propoxy-nucleosides did not support ***RNase*** ***H1*** activity. The Km and Vmax. of cleavage of RNA duplexes with full phosphorothioate oligodeoxynucleotides were compared with methoxydeoxy 'gapmers', i.e.; oligonucleotides with 2'-methoxy wings surrounding a deoxynucleotide center. Such structural modifications resulted in substantial increases in affinity, but significant redns. in cleavage efficiency. The initial rates of cleavage increased as the deoxynucleotide gap size was increased. Multiple deoxynucleotide gaps increased the Vmax. but had little effect on Km. The effects of several base modifications on the site of initial cleavage, processivity and initial rate of cleavage were also studied.

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1995:962545 Document No. 124:109876 Inhibition of splicing of wild-type and mutated luciferase-adenovirus pre-mRNAs by antisense oligonucleotides. Hodges, Dianne; ***Crooke, Stanley T.*** (Dep. Anatomy Neurobiology, Univ. California, Irvine, CA, 92717, USA). Molecular Pharmacology, 48(5), 905-18 (English) 1995. CODEN: MOPMA3. ISSN: 0026-895X. Publisher: Williams & Wilkins.

AB We report the construction, characterization, and use of luciferase reporters to test the ability of antisense oligonucleotides to inhibit RNA splicing. .beta.-Globin and adenovirus introns were inserted into a luciferase cDNA, and luciferase expression was analyzed in transiently transfected cells. The adenovirus reporter expressed large amts. of luciferase, but two .beta.-globin constructs were inactive. RNA analyses detd. that the .beta.-globin pre-mRNAs were not spliced. Mutagenesis of the .beta.-globin 5'-splice site, branchpoint, and 3'-splice site sequences to the adenovirus intron sequences promoted maximal splicing and luciferase activity; reciprocal changes in all three elements of the adenovirus intron eliminated luciferase activity. Wild-type and 3'-splice site mutated adenovirus reporters were used to det. the ability of phosphorothioate deoxy and 2'-methoxy oligonucleotides to inhibit splicing. ***RNase*** ***H*** -activating oligodeoxynucleotides were better inhibitors of wild-type adenovirus expression than were 2'-methoxy analogs. However, 2'-methoxy oligonucleotides specific for the branchpoint were more effective inhibitors of splicing of adenovirus transcript contg. the .beta.-globin branchpoint and 3'-splice site. We

suggest that pre-mRNAs with weak splice sites are potential targets for oligonucleotides that inhibit splicing by occupancy rather than cleavage of the transcripts.